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Archegonial Development and Oogenesis of the Fern *Plagiogyria euphlebia* and their Phylogenetic Significance

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ABSTRACT.—The cytological features of the cells taking part in archegonial development and oogenesis in the fern *Plagiogyria euphlebia* (Kunze) Mett. were described in detail by means of light and electronic microscopy. The archegonium develops from an initial cell, which contains dense cytoplasm in contrast to the somatic cells. Two divisions of the initial cell result in a tier of three cells. The middle of which finally develops into a neck canal cell, a ventral canal cell and an egg by two unequal divisions. During maturation, the egg cell becomes progressively isolated from the adjacent cells by forming a separation cavity, a casual wall and an egg envelope. Series sections show that a fertilization pore forms in the upper egg envelope. During maturation of the egg, the nucleus produces conspicuous evaginations. The phylogenetic relationship of the fern *P. euphlebia* is discussed according to the cytological features in oogenesis. The cytological features observed during oogenesis support the inclusion of Plagiogyriaceae among the tree-ferns as proposed from molecular analyses.

KEY WORDS.—Archegonial development, fern, oogenesis, phylogeny, *Plagiogyria euphlebia*

Recent investigations on oogenesis revealed that core-leptosporangiate ferns produce an obvious egg envelope and a fertilization pore in the mature egg (Cao *et al.*, 2009; Cao *et al.*, 2010a, b; Dai *et al.*, 2010); however, the mature egg of *Osmundaceae*, the basal-most member of leptosporangiate ferns (Smith *et al.*, 2006), does not possess a typical egg envelope and fertilization pore (Bao *et al.*, 2003; Cao *et al.*, 2012). Osmundaceae is considered a basal family within the leptosporangiate ferns with a long evolutionary history (Bell, 1986). In the bryophyte *Marchantia polymorpha* L., no extra membrane was observed around the mature egg (Zinsmeister and Carothers, 1974). It is concluded that the cytological features of the egg in oogenesis are closely related to the phylogenetic position. The sporophyte morphology supports the conclusion that the Plagiogyriaceae has a close relationship to Osmundaceae (Nayar, 1970; Mickel, 1974; Wu and Ching 1991). However, the relationships of Plagiogyriaceae to other ferns remain uncertain (Smith, 1995). Molecular data provide strong evidence for the inclusion of *Plagiogyria* within the tree ferns (Hasebe *et al.*, 1995; Pryer *et al.*, 2001; Pryer *et al.*, 2004; Smith *et al.*, 2006). In the present study, the oogenesis of the fern *Plagiogyria euphlebia* (Kunze) Mett. was studied and its phylogenetic similarity with respect to this character is discussed.

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Materials and Methods

Spores of *Plagiogyria euphlebia* were collected from plants in Wuyishan Nature Reserve of Jiangxi province, China. The spores were surface sterilized with 5% sodium hypochlorite solution for 3 min. After rinsing three times with distilled water, the spores were sown on a modified Knop's solution (0.8g $Ca(NO_3)_2 \cdot 4H_2O$; 0.2g KH_2PO_4; 0.2g KNO_3; 0.2g $MgSO_4 \cdot 7H_2O$, dissolved in 1 L distilled water), and solidified with 1.5% agar in culture dishes. These dishes were placed in an artificial climate chamber under conditions of 25°C in the light (18 h) and 20°C in the dark (6 h). After 8 to 9 weeks, archegonia had developed on the lower surface of the gametophytes just behind the growing apex.

Gametophytes bearing various archegonial stages were placed in 3% glutaraldehyde in 0.1 mol/L phosphate buffer at room temperature for 6–12 h. The specimens were subsequently washed three times with the same buffer, postfixed in 2% aqueous osmium tetroxide for 2 h, rinsed three times in buffer and embedded in Spurr's resin (SPI-Chem, USA) via a graded acetone series. Specimens were thick sectioned for the presence of the archegonia and thin-sectioned with a diamond knife on an Ultracut-E ultramicrotome (Reichert-Jung, Germany). The thick sections were stained with Toluidine blue and observed using a light microscope. The thin sections were stained with uranyl acetate and lead citrate. All specimens were observed with H-600 electron microscope (Hitachi, Japan).

Results

The initial cell.—Archegonia of *Plagiogyria euphlebia* are usually produced on the lower surface of the gametophyte just behind the growing point (Fig. 1A). The cells coming to form archegonia (the initial cell, ic) can be identified by the cylindrical shape of the cell, which possesses more cytoplasm around the central placed nucleus in contrast to the somatic cells (Fig. 1B, C). The vacuoles in the initial cell are asymmetrically distributed. One or two large vacuoles are located in the lower part, and small vacuoles lie in the upper part of the cell (Fig. 1C). The chloroplasts in the initial cell, lacking well-developed lamellae and containing little starch, are usually smaller than those in the somatic cells (Fig. 1D). Mitochondria show little difference from those in the somatic cells. Crystals are frequently seen in plastids both in the archegonial initial and the somatic cells (Fig. 1D, asterisks). Subsequently, the initial cell divides into two by a periclinal division. The upper cell becomes the neck jacket initial (Fig. 1E, c1), which contains few vacuoles. The lower cell usually contains some large vacuoles at the basal part (Fig. 1E, c2). At this stage, there are well-developed plasmodesmata between the archegonial cells and the somatic cells (Fig. 1D, F).

The primary cell.—The lower cell forms a primary cell (pc) and a basal cell by a periclinal division (Fig. 1G). The primary cell is a square with almost equivalent height and width. The nucleus is larger and the chromatin becomes more dispersed than those in the adjacent cells (Fig. 1G). The plasmodesmata are well developed between the primary cell and the upper neck initial cell (Fig. 1H), but

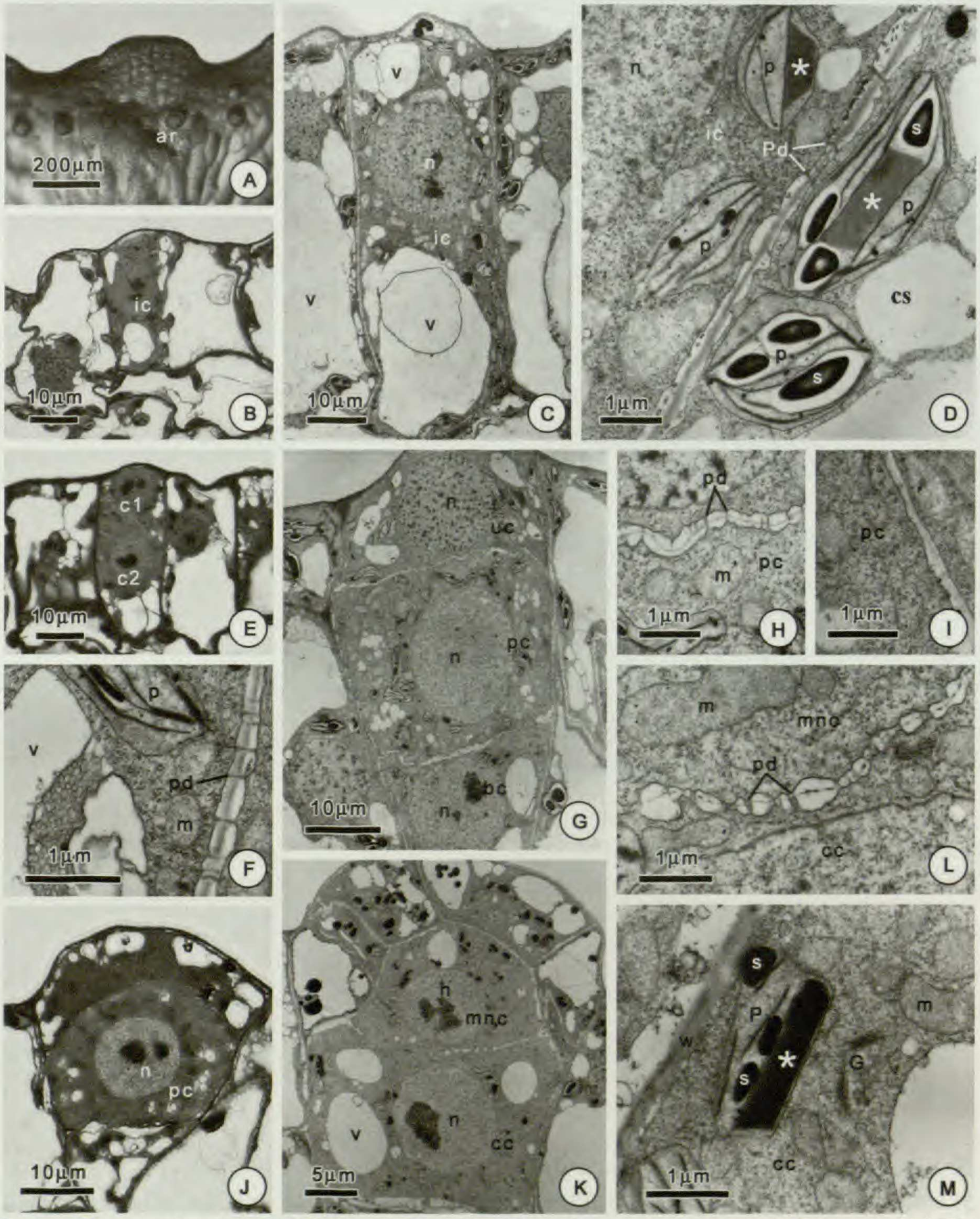

FIG. 1. Young archegonium of *Plagiogyria euphlebia* (**A**, under stereomicroscope; **B, E, J**, under LM; **The others**, under TEM). **A.** Archegonia (ar) are produced under the growing point of the prothallus; **B, C.** Initial cell (ic) possess a large nucleus and more cytoplasm around it. **D.** Plastids in the initial cell (ic) are obviously smaller and contain fewer starch grains than those in the somatic cells (cs). **E.** The initial cell divides into an upper cell (c1) and a basal cell (c2). **F.** Plasmodesmata are obvious between the basal cell and somatic cell. **G.** A tier of three cells are formed, which are the upper cell (uc), the primary cell (pc), and basal cell (bc). **H.** Plasmodesmata (pd) between the upper cell and the primary cell (pc). **I.** Plasmodesmata disappear between the primary cell and the somatic cell. **J.** The top of the primary cell (pc) bulges and the upper cell divides into four. **K.** The primary cell forms a central cell (cc) and a mononucleate neck canal cell (mnc) and by an unequal division; **M.** The central cell (cc) contains abundant organelles. G, Golgi bodies; m, mitochondrion; n, nucleus; p, plastid; pd, plasmodesmata; s, starch.

those are absent in the wall between the primary cell and the somatic cells (Fig. 1I). The organelles, including plastids and mitochondria, resemble those in previous stage. Soon, the pc enlarges and its upper surface protrudes upwards (Fig. 1J). Before division of the primary cell, the neck initial cell divides into a rosette of four cells by two anticlinal divisions (Fig. 1J).

The central cell.—The primary cell divides asymmetrically to form two cells. The cell towards the neck of the archegonium is a mononucleate neck canal cell (mnc) with little cytoplasm (Fig. 1K). The lower cell, obtaining more cytoplasm from its mother cell, is named as the central cell (cc) (Fig. 1K). A conspicuous feature of this stage and also at subsequent stages is that there are well-developed plasmodesmata between the neck canal cell and the central cell (Fig. 1L). Organelles in the central cell resemble those in the previous stage (Fig. 1M). Sometimes, a few large vacuoles can be seen in the cytoplasm (Fig. 1K).

The newly formed egg.—The central cell also divides asymmetrically to form a small ventral canal cell (vcc) and a large egg cell, which has most of the cytoplasm (Fig. 2A). Soon after the egg is formed, the nucleus of the mononucleate neck canal cell divides into two, without cell wall formation between the two nuclei, which resulting in a binucleate neck canal cell (ncc) (Fig. 2B). The newly formed egg and the neck and ventral canal cells are closely appressed to the archegonial jacket cells (Fig. 2B). Well-developed plasmodesmata connect the ventral canal cell and the neck canal cell (Fig. 2C), and also connect the egg and the ventral canal cell, but these are absent between the inner three cells and the jacket cells (Fig. 2D). The nucleus of the young egg is spherical and typical sections show it contains one or two nucleoli (Fig. 2A, B). The newly formed egg contains abundant vesicles in the cytoplasm (Fig. 2B). Plastids contain fewer starch grains and lamellae than previous stages (Fig. 2D). Organelles in the VCC and NCC (Fig. 2C) resemble those in the egg (Fig. 2D).

Egg maturation.—Egg maturation undergoes remarkable cytological changes including the formation of a separation cavity, an osmiophilic egg envelope, a fertilization pore and prominent nuclear evaginations. In the early stage of egg maturation, the most conspicuous feature in oogenesis is formation of separation cavity (sc). Serial sections show that the separation cavity initially begins to form around the periphery of the upper surface of the egg (Fig. 2E). The separation cavity expands centripetally and the connection region is correspondingly reduced. However, a pore region, which is about 2–3 μm, persistently connects the egg and the ventral canal cell (Fig. 2E, arrow; Fig. 2F, G). Well-developed plasmodesmata can be seen in the pore region (Fig. 2G). Sections stained with toluidine blue show that there is a deeply stained cell wall (Fig. 2E, arrowhead) between the egg and the ventral canal cell except in the pore region (Fig. 2E, arrow). This thickened cell wall is closely appressed to the ventral canal cell (Fig. 2F, arrowheads; Fig. 2G). In the lower part of the egg, a narrow separation cavity is formed between the plasmolemma and the cell wall (Fig. 2H). At this stage, the endoplasmic reticula and Golgi bodies become more observable than previous stage both in the egg and in the canal cells (Fig. 2G). Vesicles are mainly distributed in the upper part of the egg cytoplasm. The egg nucleus now becomes somewhat ellipsoid in shape with a depressed upper surface (Fig. 2E, F).

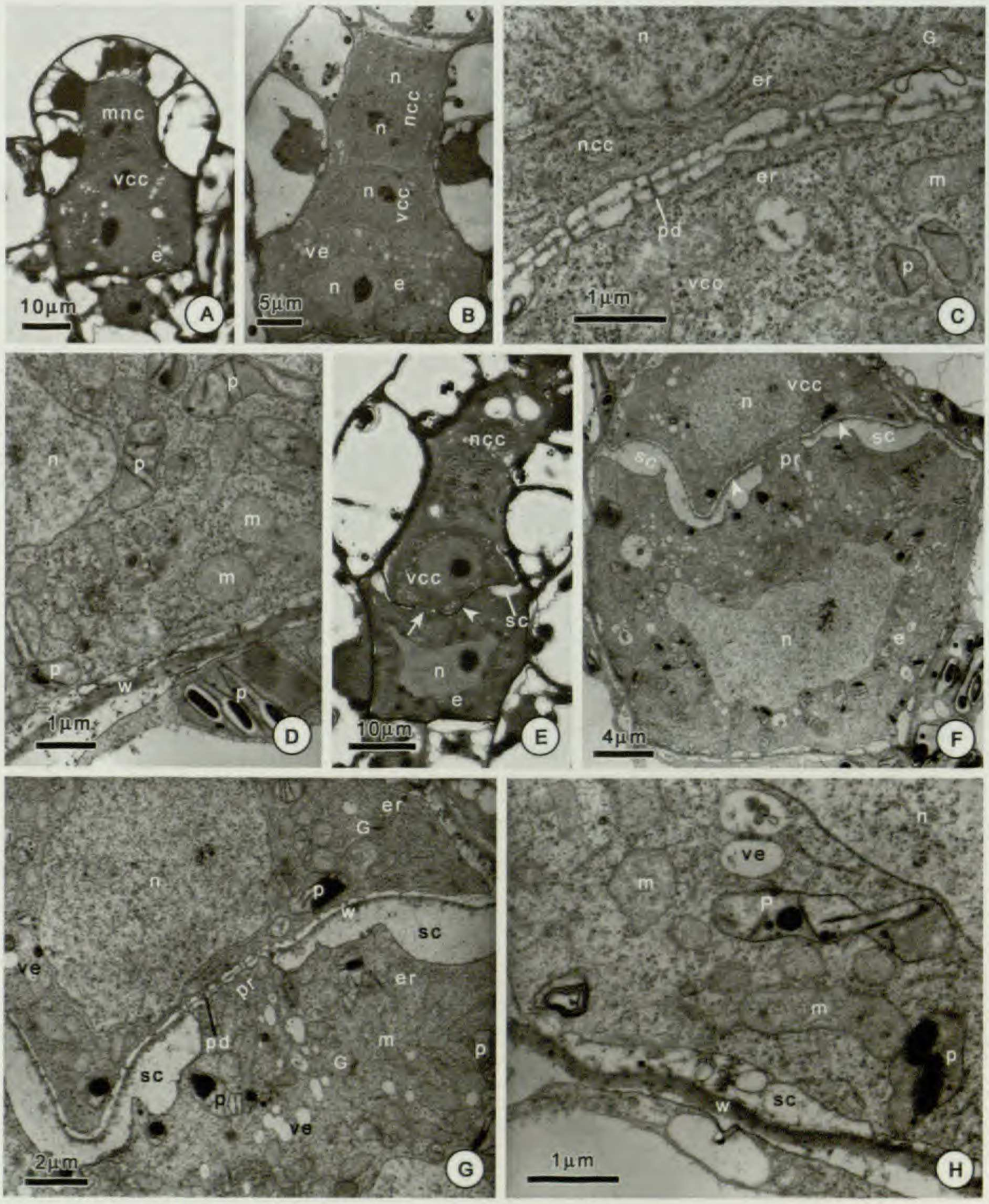

FIG. 2. Early stage of the egg development (**A**, **E**, under LM; **The others**, under TEM). **A.** An egg (e) and a ventral canal cell (vcc) is formed by an unequal division of the central cell; **B.** A binuclear elongated neck canal cell (ncc) is formed; **C.** Magnification of Fig. 2B showing the plasmodesmata between the vcc and the ncc; **D.** Part of the egg showing the organelles in the cytoplasm; **E.** Maturing egg stage, a separation cavity (sc) begins to form above the egg. A casual cell wall (arrowhead) between the egg and the vcc becomes obvious thickened. A pore region (arrow) connects the egg and vcc; **F.** The separation cavity (sc) forms around the egg. The casual cell wall (arrowheads) lies closely to the vcc. The pore region (pr) connects the egg and the vcc; **G.** Magnification of Fig. 2F showing the pore region (pr). **H.** The separation cavity (sc) in the lower part of the egg. G, Golgi bodies; m, mitochondrion; mnc, mononucleate neck canal cell; n, nucleus; p, plastid; pd, plasmodesmata; ve, vesicle; w, cell wall.

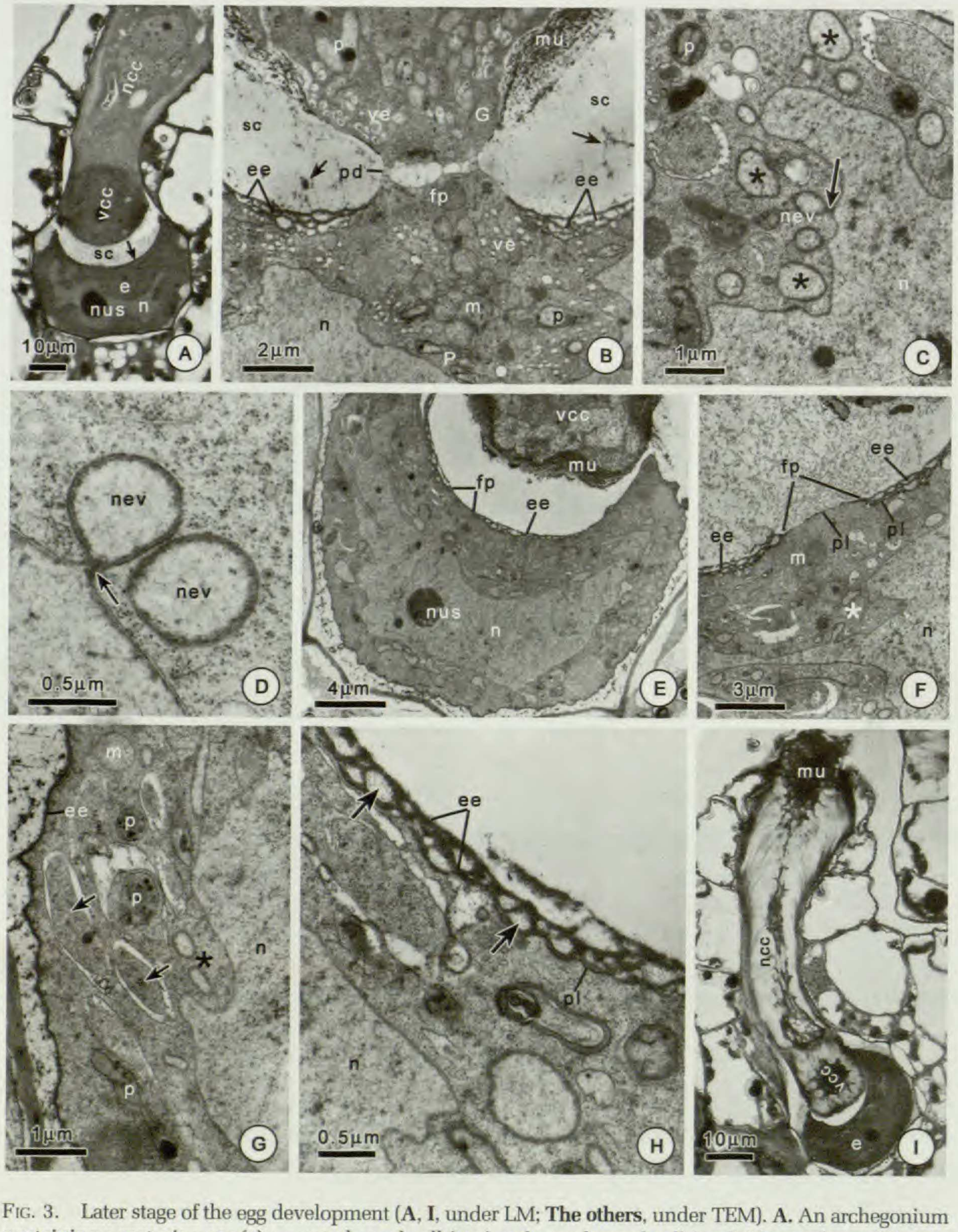

FIG. 3. Later stage of the egg development (**A**, **I**, under LM; **The others**, under TEM). **A.** An archegonium containing a maturing egg (e), a ventral canal cell (vcc) and a neck canal cell (ncc). The nucleus of the egg becomes highly irregular; **B.** The egg envelope (ee) covers the upper surface of the egg except in the connection region. Here a fertilization pore (fp) is formed; **C, D.** Nuclear evaginations (nev), some of which connected to the main body of the nucleus by a narrow isthmus (arrows); Some others are probably detached from the nucleus body (asterisks); **E.** The mature egg has a fertilization pore (fp). The vcc is dissociated from the egg. **F.** Another egg showing the fertilization pore (fp). Asterisk indicates the nuclear evaginations; **G.** Vacuolated cytoplasm (arrows) is often seen in the mature egg. Nuclear evaginations (asterisk) become ellipsoid; **H.** The egg envelope (ee) shows a reticular structure. Light-stained spaces

Subsequently, a special egg envelope is formed around the egg (Fig. 3A, arrow), which is accompanied by the disappearance of the cell wall between the egg and the ventral canal cell seen in previously stage (Fig. 3B). The upper egg envelope is much thicker and more conspicuous (Fig. 3B). The egg is still connected with the ventral canal cell in the pore region and no egg envelope is formed in this region (Fig. 3B), which leads to formation of a fertilization pore. In the separation cavity, amorphous materials are obvious above the egg envelope (Fig. 3B, arrows). The organelles in the egg cytoplasm differ greatly from those in the previous stage. Plastids degenerate further, but mitochondria are more prominent (Fig. 3B, C). In the ventral canal cell, vesicles and Golgi bodies increase, whilst mucilaginous materials accumulate around this cell (Fig. 3B, Mu).

At the middle stage of the egg development, the original ellipsoidal nucleus of the egg develops into an irregular cup-shape (Fig. 3A) and its surface produces numerous sac-like evaginations (Fig. 3C, D, nev). These are usually between 0.5–0.8 μm in diameter and remain connected to the main body of the nucleus via narrow isthmuses (Fig. 3C, D, arrow). Sacs similar to the nuclear evaginations, but without connection to the nucleus, appear in the cytoplasm of the egg (Fig. 3C, asterisks). The matrix of the evaginations resembles that of the main body of the nucleus (Fig. 3C, D). But the double membranes of the nuclear envelope cannot be recognized clearly (Fig. 3D).

The mature egg.—When the egg matures, the ventral canal cell is usually detached from the egg at the region of fertilization pore (Fig. 3E, F). The transverse diameter of the fertilization pore reaches 3 μm. No egg envelope covers this region. The plasmalemma covers the fertilization pore and the egg envelope lies outside of the plasmalemma on the remainder of the egg (Fig. 3F). The nucleus of the egg remains irregular in outline and evaginations can be seen here and there (Fig. E). However, most of these evaginations may have become ellipsoid in shape (Fig. 3F, G, asterisks). A most obvious change occurring in the cytoplasm of the egg is the appearance of the vacuolated organelles in the upper cytoplasm of the matured egg (Fig. 3G, arrows). Plastids, without any starch grains and lamellae, only can be identified by plastoglobuli (Fig. 3G). The egg envelope in the upper surface of the egg is especially obvious, which shows a reticular structure (Fig. 3H). The thickness of the upper egg envelope almost reaches 0.5 μm (Fig. 3H), but in the lower part the width of the egg envelope is only about 50–60 nm (Fig. 3G). As the egg matures completely, the canal cells degenerate. Most of the cytoplasm of the canal cells has decomposed into an amorphous mucilaginous material, which moves towards the opening of the archegonium when met with water (Fig. 3I).

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(arrows) are seen in it; **I.** Mature archegonium with a mature egg and degenerated vcc and ncc. Mucilaginous material (mu) move towards the opening of the archegonium. G, Golgi bodies; m, mitochondrion; n, nucleus; nus, nucleolus; p, plastid; pd, plasmodesmata; pl, plasmalemma; sc, separation cavity; ve, vesicle.

Discussion

Early development of the archegonium.—The stages of archegonial development of *Plagiogyria euphlebia* is similar to that described for other derived ferns described previously (Bell and Mühlethaler, 1962a; Yang *et al.*, 2009; Dai *et al.*, 2010). However, the detailed ultrastructural features of the initial cell, the primary cell and central cell were lacking. The present investigation shows that the cells taking part in oogenesis usually possess much more cytoplasm. The plastids in these cells rarely contain starches, which is the typical feature of the meristematic cells. Furthermore, large polyhedral crystals in the plastids, previously reported in some angiosperms (Williams, 1974), are observed in fern cells for the first time. Vacuoles in the initial cell are distributed asymmetrically, which undoubtedly influence the polarity of the initial cell and finally result in three functionally different cells in an axial tier. The uppermost cell develops into the neck jacket cells of the archegonium. The middle cell finally develops into the egg and the two canal cells and the lowermost cell becomes a somatic cell and participates formation of the basal jacket cells in the later stage of the egg development.

The egg envelope and fertilization pore.—As in *Ceratopteris* (Cao *et al.*, 2009, 2010a) and *Adiantum* (Cao *et al.*, 2010b), *Plagiogyria euphlebia* also forms a prominent egg envelope and a fertilization pore. The egg envelope of the mature egg of *P. euphlebia* resembles those of *Pteridium aquilinum* (L.) Kuhn (Duckett and Bell, 1972), *Histiopteris incise* (Thunb.) J.Sm. (Bell, 1980), *Athyrium filix–femina* (L.) Roth (Fasciati *et al.*, 1994), *Dryopteris crassirhizoma* Nakai (Bao *et al.*, 2005) in structure, but differs somewhat from that of *Ceratopteris* and *Adiantum*, in which the egg envelope is composed of multilayered membranes (Cao *et al.*, 2008; Lopez-Smith and Renzaglia, 2008; Cao *et al.*, 2010a). In *Ceratopteris*, the egg envelope is believed to be formed by attachment of endoplasmic reticula (Cao *et al.*, 2008). However, endoplasmic reticula are rarely discovered in the maturing egg of *P. euphlebia*. The formation of the egg envelope may occur in another way. The osmiophilic amorphous materials in the separation cavity seem to be used to form the egg envelope on the outer surface of the egg plasmalemma. The structural difference in the egg envelope may have some significance in the classification of ferns. The ventral canal cell participates in the formation of the fertilization pore as suggested in *Ceratopteris* (Cao *et al.*, 2010a). The persistent connection of the egg and vcc in the pore region results in no deposition of the egg envelope on this region and finally forms a fertilization pore.

Nuclear behavior and evagination.—*Plagiogyria euphlebia* produces an irregular nucleus and obvious nuclear evaginations in the maturing egg, which resemble the derived ferns *Pteridium aquilinum* (Bell and Mülethaler, 1962b; Bell, 1972; Bell and Duckett, 1976; Bell, 1983), *Dryopteris filix-mas* (L.) Schott (Cave and Bell, 1975), *Histiopteris incisa* (Bell, 1980), *Dryopteris crassirhizoma* (Bao *et al.* , 2005) and *Adiantum* (Cao *et al.* , 2010b). In *Ceratopteris thalictroides* (L.) Brongn., the nucleus also becomes highly irregular, but it does not produce evaginations during oogenesis. The older fern lineage *Osmunda* possesses a regular nucleus (Bao *et al.*, 2003; Cao *et al.*, 2012). Therefore, the nuclear behavior and evaginations may have some significance in assessing the phylogenetic

affiliation of ferns. The derived ferns tend to produce more complicated evaginations.

Plasmodesmata diminishing and isolation of the egg.—The changes of the plasmodesmata are remarkable during oogenesis. The present investigation shows that the cells taking part in oogenesis, including the initial cell, the primary cell, the central cell and the egg cell, progressively lost the plasmodesmatal connections with their adjacent cells. For the egg, isolation is strengthened by forming a temporary cell wall between the egg and the vcc, and a permanent egg envelope around the egg. The biological significance of the isolation of the cells taking part in oogenesis most probably ensures the independent development of the sex cells. Bell and Duckett (1976) also indicated that the cells taking part in oogenesis in *Pteridium aquilinum* are progressively isolated from the remainder of the gametophyte.

The cytological features of oogenesis and their phylogenetic significance.—The family Plagiogyriaceae is usually considered to be basal based on morphology of the sporophytes. It was suggested that Plagiogyriaceae is closely related to the family Osmundaceae and may have evolved from a common ancestor (Nayar, 1970; Mickel, 1974). Molecular data do not support the conclusion that the Plagiogyriaceae is closely related to the Osmundaceae, but support the inclusion of the Plagiogyriaceae within the tree ferns (Hasebe *et al.*, 1995; Pryer *et al.*, 2001; Pryer *et al.*, 2004; Smith *et al.*, 2006). The present investigation shows that in *Plagiogyria euphlebia* the egg's cytological features, including the egg envelope and nuclear evaginations, are identical to the *Cibotium barometz* (L.) J.Sm. (Cao, unpublished data), which is a member of the tree ferns (Pryer *et al.*, 2001; Pryer *et al.*, 2004). *Plagiogyria euphlebia* and other polypodiaceous ferns usually possess a prominent egg envelope and fertilization pore in the mature egg (Cao *et al.*, 2009, 2010a, b; Dai *et al.*, 2010); however, *Osmunda* does not possess a typical egg envelope and fertilization pore (Bao *et al.*, 2003; Cao *et al.*, 2012). These observations support the inclusion of *Plagiogyria* among the tree ferns.

Acknowledgments

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Literature Cited

Bao, W. M., J. G. Cao and S. J. Dai. 2003. Ultrastructure of oogenesis in *Osmunda cinnamomea* var. *asiatica*. Acta Bot. Sin. 45:843–851.

Bao, W. M., Q. He, Q. X. Wang, G. W. Tian and J. G Cao. 2005. Ultrastructure of Oogenesis *in Dryopteris crassirhizoma* Nakai. J. Integr. Plant Biol. 47:201–213.

Bell, P. R. 1972. Nucleocytoplasmic interaction in the eggs of *Pteridium aquilinum* maturing in the presence of thiouracil. J. Cell Sci. 11:739–755.

Bell, P. R. 1980. Nucleocytoplasmic interaction during maturation of the egg of the fern *Histiopteris incisa* (Thunb.) J. Smith. Ann. Bot. 45:475–481.

Bell, P. R. 1983. Nuclear bodies in the maturing egg cell of a fern, *Pteridium aquilinum*. J. Cell Sci. 60:109–116.

Bell, P. R. 1986. Features of egg cells of living representatives of ancient families of ferns. Ann. Bot. 57:613–621.

Bell, P. R. and J. G. Duckett. 1976. Gametogenesis and fertilization in *Pteridium*. Bot. J. Linn. Soc. 73:47–78.

Bell, P. R. and K. Mühlethaler. 1962a. A membrane peculiar to the egg in the gametophyte of *Pteridium aquilinum*. Nature 195:198.

Bell, P. R. and K. Mühlethaler. 1962b. The fine structure of the cells taking part in oogenesis in *Pteridium aquilinum* (L.) Kuhn. J. Ultrastruct Res. 7:452–466.

Cao, J. G., X. F. Dai and Q. X. Wang. 2012. Cytological features of oogenesis and their evolutionary significance in the fern *Osmunda japonica*. Sex. Plant Reprod. DOI: 10.1007/s00497-011-0179-7.

Cao, J. G., Q. X. Wang and W. M. Bao. 2010a. Formation of the fertilization pore during oogenesis of the fern *Ceratopteris thalictroides*. J. Integr. Plant Biol. 52:518–527.

Cao, J. G., Q. X. Wang, X. L. Dai and J. G. Duckett. 2010b. Ultrastructural observations of oogenesis in the fern *Adiantum flabellulatum* L. (Adiantaceae). Amer. Fern J. 100:93–102.

Cao, J. G., N. Y. Yang and Q. X. Wang. 2008. Observations on the formation and ultrastructure of the egg membrane in the fern *Ceratopteris thalictroides* (L.) Brongn. Acta. Bot. Yunnan. 30:543–548.

Cao, J. G., Y. N. Yang and Q. X. Wang. 2009. Ultrastructure of the mature egg and fertilization in the fern *Ceratopteris thalictroides* (L.) Brongn. J. Integr. Plant Biol. 51:243–250.

Cave, C. F. and P. R. Bell P. R. 1975. Evidence for the association of acyl transferases with the production of nuclear evaginations in maturing eggs of the fern *Dryopteris filix-mas*. Histochemistry 44:57–65.

Dai, X. F., N. Jiang, J. G. Cao and Q. X. Wang. 2010. Primary Study on the Archegonium Formation and Oogenesis in the Fern *Phymatosorus hainanensis*. Bull. Bot. Res. 30:411–415.

Duckett, J. G. and P. R. Bell. 1972. Studies on fertilization in archegoniate plants II. Egg penetration in *Pteridium aquilinum* (L.) Kuhn. Cytobiologie 6:35–50.

Fasciati, R., J. Schneller, V. Jenni and U. P. Roos. 1994. Fertilization in the fern *Athyrium filix-femina* (Pterophyta): II. Ultrastructure. Crypt. Bot. 4:356–367.

Hasebe, M., P. G. Wolf, K. M. Pryer, J. R. Manhart, N. Murakami, E. H. Crane, C. H. Haufler and W. D. Hauk. 1995. Fern phylogeny based on *rbcL* nucleotide sequence. Amer. Fern J. 85:134–181.

Lopez-Smith, R. and K. Renzaglia. 2008. Sperm cell architecture, insemination, and fertilization in the model fern, Ceratopteris richardii. Sex. Plant Reprod. 21:153–167.

Mickel, J. T. 1974. Phelytic lines in the modern ferns. Ann. Missouri. Bot. Garden. 61:474–482.

Nayar, B. K. 1970. A phylogenetic classification of the homosporous ferns. Taxon 19:229–236.

Pryer, K. M., H. Schneider, A. R. Smith, R. Cranfill, P. G. Wolf, J. S. Hunt and S. D. Sipes. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature 409:618–622.

Pryer, K. M. and P. G. Wolf. 2004. Phylogeny and evolution of ferns (Monilophytes) with a focus on the early leptosporangiate divergenes. Amer J. Bot. 91:1582–1598.

Smith, A. R. 1995. Non-molecular phylogenetic hypotheses for ferns. Amer. Fern J. 85:104–122.

Smith, A. R., K. M. Pryer, E. Schuettpelt, P. Korall, H. Schneider and P. G. Wolf. 2006. A classification for extant ferns. Taxon 55:705–731.

Williams, E. 1974. Fine structure of vascular and epidermal plastids of the mature Maize Leaf. Protoplasma 79:395–400.

Wu, S. H. and R. C. Ching. 1991. Fern families and genera of China. Beijing, Science press.

Yang, N. Y., J. G. Cao and Q. X. Wang. 2009. Formation and Development of Archegonium in the fern *Ceratopteris thalictroides* (L.)Brongn. J. Wuhan Bot. Res. 27:569–573.

Zinsmeister, D. D. and Z. B. Carothers. 1974. The Fine Structure of Oogenesis in *Marchantia polymorpha*. Amer. J. Bot. 61:499–512.